

AMENDMENTS TO THE CLAIMS

The following is a marked-up version the Claims pursuant to 37 C.F.R. §1.121 (c)(1)(ii) with instructions and markings showing changes made herein to the previous version of record of the Specification and Claims. Underlining denotes added text while ~~strikeout~~ denotes deleted text.

Claims 1-48 (previously cancelled)

Claim 49. (previously added) A method for preparing an evolved microorganism comprising the steps of:

- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein said at least one heterologous protein is an enzyme;
- b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type; and
- c) restoring said evolved microorganism to a wild type mutation rate.

Claim 50. (previously added) The method of Claim 49, wherein said at least one heterologous protein is a hydrolase.

Claim 51. (previously added) The method of Claim 50, wherein said hydrolase is selected from the group consisting of proteases, esterases, lipases, phenol oxidase, permeases, amylases, pullulanases, cellulases, glucose isomerase, laccases, and protein disulfide isomerases.

Claim 52. (previously added) The method of Claim 49, wherein said microorganism comprises at least one copy of said mutator gene in its chromosome and said step of restoring said evolved microorganism to wild-type mutation rate comprises excision of said mutator gene.

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Claim 53. (amended) The method of Claim 52, wherein said mutator gene comprises at least one gene selected from the group consisting of ~~mutD, mutT, mutY, mutM, mutH, mutL, mutS, mutU~~, *mutD* mutations, *mutT* mutations, *mutY* mutations, *mutM* mutations, *mutH* mutations, *mutL* mutations, *mutS* mutations, *mutU* mutations, and homologues of *mutD*, *mutT*, *mutY*, *mutM*, *mutH*, *mutL*, *mutS*, and *mutU*.

Claim 54. (previously added) The method of Claim 52, wherein said mutator gene comprises *mutD* mutations selected from the group of *mutD* mutations set forth in Table 1.

Claim 55. (previously added) A method for preparing an evolved microorganism comprising the steps of:

- a) obtaining a microorganism comprising at least one heterologous mutator gene and at least one introduced nucleic acid encoding at least one heterologous protein, wherein said at least one heterologous protein is an enzyme necessary for an enzymatic pathway;
- b) culturing said microorganism for at least 20 doublings under conditions suitable for selection of an evolved microorganism, wherein said heterologous mutator gene generates a mutation rate of at least 5-100,000 fold relative to wild type; and
- c) restoring said evolved microorganism to a wild type mutation rate.

Claim 56. (previously added) The method of Claim 55, wherein said enzyme is selected from the group consisting of reductases and dehydrogenases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of ascorbic acid or ascorbic acid intermediates.

Claim 57. (previously added) The method of Claim 55, wherein said enzyme is selected from the group consisting of glycerol dehydratase and 1,3-propanediol dehydrogenase, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of 1,3-propanediol, 1,3-propanediol precursors, and 1,3-propanediol derivatives.

Claim 58. (previously added) The method of Claim 55, wherein said enzyme is selected from the group consisting of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatases, and further wherein said enzymatic pathway results in the production of at least one compound selected from the group consisting of glycerol and glycerol derivatives.